Spermatozoal Ultrastructure in Dendrobranchiata (Crustacea, Decapoda): Taxonomic and Phylogenetic Considerations

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ABSTRACT

This chapter reviews the diverse spermatozoal patterns found to date in Dendrobranchiata, including the first ultrastructural descriptions of solenocerid and sergestid spermatozoa. Some characters are analysed from a spermiocladistic perspective and a tentative phylogram is presented where the dendrobranchiate sperm forms are related to phyletic arrangements inferred from holomorphological studies. Evolutionary relationships within Eucarida are discussed with respect to the various sperm morphologies.

RÉSUMÉ

Ultrastructure du spermatozoïde chez les Dendrobranchiata (Crustacea, Decapoda): considérations taxonomiques et phylogénétiques

Ce chapitre synthétise nos connaissances sur les différentes morphologies des spermatozoïdes rencontrées jusqu'ici chez les Dendrobranchiata, y compris les premières descriptions ultrastructurales du spermatozoïde d'un Solenoceridae et d'un Sergestidae. Quelques caractères sont analysés dans une perspective spermiocladistique et un essai de phylogramme est présenté, dans lequel les morphologies des spermatozoïdes des Dendrobranchiata sont mises en relation avec les arrangements phylogéniques issus des études holomorphologiques. Les relations évolutives chez les Eucarides sont discutées en relation avec les différentes morphologies des spermatozoïdes.

The almost limitless morphological diversity of animal spermatozoa has inspired an extensive amount of research work in diverse biological fields. Specifically, in recent years sperm ultrastructure has become a useful tool in studies on taxonomy and phylogeny, since it proves effective in resolving problems which escape conventional somatic analyses [25, 30]. The shape and inner organization of a given sperm cell is definitely characteristic of the species that produces it; that is, the spermatozoal ultrastructure constitutes a distinctive character of identity for every animal species. Correspondingly, it is obvious that the evolution of a spermatozoon runs parallel to the evolution of the corresponding species from which it comes. On this basis, an increasing number of works have been conducted to establish a congruent relationship between the sperm

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ultrastructure and the evolution and phylogeny of many animal taxa. JAMIESON [25] coined the terms "spermiocladistics" and "spermiotaxonomy" in reference to the application of sperm ultrastructure to phylogenetic and taxonomic studies.

The astonishing diversity of forms that animal spermatozoa can adopt is well exemplified by the crustaceans, where a wide range of sperm types vary from the aquasperm-like (plesiomorphic) remipedian spermatozoon, through the amoeboid and acrosome-less forms present in several taxa, to the aflagellate, either "unistellate" or "multistellate", decapod sperm [31]. The extreme strangeness of these spermatozoa aroused the curiosity of early spermatologists. Light microscopical reports on crustacean spermatozoa are relatively abundant and precocious, some of them including phylogenetic analyses based on the sperm types of decapods (see review by FELGENHAUER & ABELE [19]). With the development of electron microscopy, the opportunity to examine in detail subcellular structures opened the range of possibilities to utilize sperm morphology in reconstruction of crustacean phylogeny. Three recent reviews have painstakingly updated the knowledge on the sperm ultrastructure in decapods [19, 31, 39]. These revisions report the traditional tendency to classify the different decapod sperm morphologies into two categories, following early classifications of the Decapoda into Natantia and Reptantia. Thus, a supposedly uniform sperm plan, referred to as the "unistellate spermatozoon", was thought to be shared by the suborder Dendrobranchiata and the pleocyematan infraorder Caridea (formerly grouped in the Natantia along with the Stenopodidea), the "multistellate spermatozoon" being typical of the rest of the representatives of the suborder Pleocyemata (former Reptantia) [6]. Most recent studies, however, have provided additional information which recommends reconsideration of this view. Actually, the general unistellate sperm pattern appears to encompass two significantly distinct sperm structures [44]. Furthermore, other sperm morphologies present in species of Dendrobranchiata do not fit into either of the two traditional categories of decapod spermatozoa ([13], MEDINA, other chapter in this book [45]).

TABLE 1. — List of Dendrobranchiata for which sperm ultrastructure is known

Family	Species	Reference
Penaeidae	*Parapeneus longirostris* (Lucas, 1846)	[44]
	Peneopsis serrata Bate, 1881	[47]
	Penaeus aztecus Ives, 1891	[9]
	Penaeus japonicus Bate, 1888	[48, 50]
	Penaeus kerathurus (Forskål, 1775)	[48]
	Penaeus setiferus (Linnaeus, 1767)	[18, 39]
	Penaeus vannamei Boone, 1931	[15, 39]
	Trachypeneus similis (Smith, 1885)	[39]
Sicyonidae	*Sicyonia brevirostris* Stimpson, 1874	[6]
	Sicyonia carinata (Brünnich, 1768)	[47]
	Sicyonia ingentis (Burkenroad, 1938)	[37, 60]
Aristeidae	*Aristaeomorpha foliacea* (Risso, 1827)	[45]
	Aristeus antennatus (Risso, 1816)	[13, 14, 45]
Solenoceridae	*Solenocera membranacea* (Risso, 1816)	[This study]
Sergestidae	*Sergestes arcticus* Kroyer, 1855	[This study]

Spermatozoal ultrastructure is well known for numerous species of reptant crustaceans, notably brachyurans. In a series of exemplary works, JAMIESON and co-workers [22, 27-29, 32-34] (see also JAMIESON, this volume) have applied spermiocladistics in this group with considerable success. However, the amount of information available on spermatozoal ultrastructure in Dendrobranchiata is more limited and mostly deals with the genera *Penaeus* and *Sicyonia* (Table 1). Wide gaps in the current knowledge of the ultrastructure of spermatozoa in Dendrobranchiata render it impracticable to perform a complete parsimony analysis of the various families and the phylogenetic relationships between them and with other decapod taxa. Nevertheless, spermiocladistic criteria may well be applied to phylogeny and taxonomy of the suborder. This chapter summarizes pre-existing information and contributes original observations, tentatively aiming at establishing valid foundations for spermiotaxonomy and spermiocladistics in the Dendrobranchiata that may be useful in forthcoming studies on this crustacean taxon.

Dendrobranchiate spermatozoal patterns

Thus far, ultrastructural descriptions of spermatozoal morphologies are available for representatives of three of the four families of the superfamily Penaeoidea: Penaeidae, Sicyonidae and Aristeidae (see Table 1 for list of species investigated). This previous information is here augmented with observations on the sperm of *Solenocera membranacea* as a representative of the family Solenoceridae. A brief ultrastructural description of the sperm of *Sergestes arcticus* is also provided in order to include a member of the superfamily Sergestoidea in support of general phylogenetic considerations. In aspects of systematics, I will here follow the classification proposed by BOWMAN & ABELE [5], whereas the spelling of taxa, and particularly that of names derived from *Pen(a)eus*, will be the one used and recommended by SCHRAM [59].

Family Penaeidae. Within the Dendrobranchiata, penaeid sperm have been the most extensively studied in terms of the number of species examined. These include five *Penaeus* species (*P. setiferus*, *P. vannamei*, *P. aztecus*, *P. japonicus* and *P. kerathurus*), *Trachypeneus similis*, *Parapeneus longirostris* and *Peneopsis serrata* (Table 1). In gross morphology, the peneid spermatozoon basically consists of a subspheroidal or ovoid main body and a spike. The main body comprises the central nuclear region, a cytoplasmic band surrounding it posterolaterally, and the acrosomal cap, which overlies the nuclear region anteriorly and is prolonged into a tapering spike (Fig. 1a, b). Both spike and acrosomal cap make up a membrane-bound acrosomal vesicle, with heterogeneous contents, which is directly invested by the plasma membrane. In particular, the spike morphology and substructure vary markedly from species to species. The whole acrosomal complex is completed with the subacrosomal region, which is quite simple in this family, merely containing a sparse flocculent material between the chromatin and acrosomal cap.

The sperm of *Parapeneus longirostris* and *Peneopsis serrata* have a central protuberance at the concave side of the acrosomal cap immediately opposite the spike (Figs 1b, 2h, i). This supposed synapomorphy is consistent with the close phylogenetic proximity of the genera *Parapeneus* and *Peneopsis*, both grouped together by BURKENROAD [8] within the tribe Parapeneini, which also includes *Artemesia* and *Metapeneopsis*. Confirmation of such a structure in the latter genera would strengthen phylogenetic unity of this taxon.

As in all dendrobranchiate species whose spermatozoon has been ultrastructurally studied, the nuclear region of peneid sperm consists of a non-membrane-bound, filamentous chromatin mass. Posterolaterally, the chromatin is surrounded by a band of cytoplasm which contains membrane lamellae, vesicles and mitochondria-like bodies, but lacks centrioles and microtubules. Within the Dendrobranchiata, the sperm of *Penaeus japonicus* are exceptional in that they exhibit several microtubule bundles in the cytoplasm [48]. The microtubules appear in primary spermatocytes of *P. japonicus* and are retained through spermiogenesis to the mature spermatozoon (personal observation). In other peneid species (e.g. *Penaeus kerathurus*, *Parapeneus longirostris*), microtubules are absent from all spermatogenetic stages.

Recent molecular studies [51] have revealed extensive genetic differences between species of *Penaeus* which have not been accompanied by substantial evolutionary morphological changes. This is congruent with the occurrence of diverse species-specific dissimilarities leading to different ultrastructure of sperm in the genus *Penaeus* and in general in the Penaeidae, and confirms the taxonomic validity of the sperm morphology in the Dendrobranchiata.

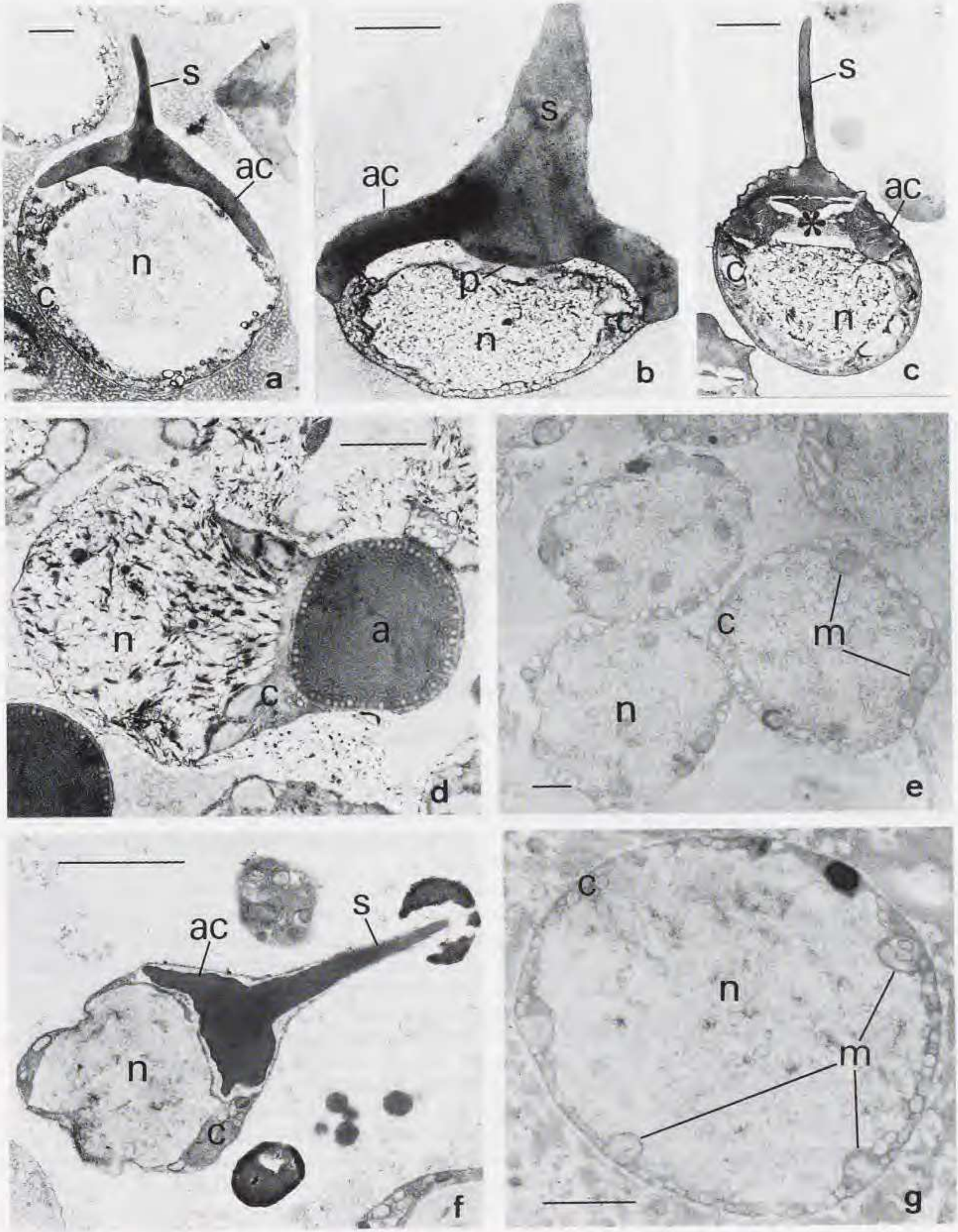
Family Sicyonidae. Ultrastructural data have been reported for three *Sicyonia* species: *S. brevirostris*, *S. carinata* and *S. ingentis* (Table 1). The inner morphological organization of the spermatozoon is very similar in *S. ingentis* [37] and *S. carinata* [47]. In general, as in Penaeidae, the sperm consist of an acrosomal vesicle (formed by the spike and acrosomal cap), subacrosomal region, and nuclear region surrounded by a cytoplasmic band (Fig. 1c). Anteriorly, the acrosome and plasma membranes are closely joined. As a taxonomically significant difference, the spike of *S. ingentis* is spiralled, whereas that of *S. carinata* is smooth. The plesiomorphies (1) absence of nuclear envelope and (2) perinuclear cytoplasmic band (containing small and large vesicles and lacking microtubules) are also found in this sperm type. Nevertheless, the highly elaborate subacrosomal region (comprising diverse distinct structures) [37, 47] appear to be a clear autapomorphy of the family Sicyonidae. Compared to the spermatozoa of the other dendrobranchiate families, the acrosomal vesicle shows the apomorphic character that the posterior membrane of the acrosomal cap is intricately folded in a ring of convoluted membrane pouches or digitations [37, 47] (Fig. 1c).

In a long series of valuable works, CLARK and co-workers have described morphological details of the acrosome reaction in *S. ingentis* [10-12, 20, 21, 24, 62]. These accounts reveal the role played by each of the spermatozoal components during fertilization, hence they greatly aid understanding of the biological significance of the acrosomal structures in dendrobranchiates.

Family Aristeidae. The relatively high ultrastructural homogeneity found within the Penaeidae and Sicyonidae is not seen in the Aristeidae. Studies of *Aristeus antennatus* [13, 14, 45] and *Aristaeomorpha foliacea* [45] indicate the existence in the family of at least two different ultrastructural sperm plans that are in turn discordant with the peneid-sicyoniid assemblage. The *A. antennatus* (Figs 1d, 2c) sperm type exhibits diverse peculiarities in comparison with the other Dendrobranchiata. First, its spherical acrosome does not cap the nuclear region and lacks both spike and subacrosomal region; the inner arrangement of the acrosomal contents is complex and different from that of any other known dendrobranchiate spermatozoon. Secondly, the cytoplasm does not constitute a band around the filamentous chromatin mass, but is accumulated in a collar between the acrosome and nuclear region, enclosing mitochondria-like vesicles and membrane lamellae. Consequently, most of the chromatin is bounded directly by the plasma membrane, since the nuclear region is, as in all dendrobranchiates, not membrane-bound. I agree with DEMESTRE & FORTUÑO [13] that the basic sperm structure of *A. antennatus* resembles that of spiny lobsters, *Panulirus* spp. [61], although with the highly significant absence of the typically reptantian radial arms, which suggests parallelism rather than a close phylogenetic relationship.

The *Aristaeomorpha foliacea* sperm type (Figs 1e, 2b), lacking the acrosome, also differs from the dendrobranchiate unistellate spermatozoal morphology. It consists of a central nuclear region entirely surrounded by the plesiomorphic cytoplasmic band, which includes membrane lamellae, small peripheral vesicles and mitochondria-like bodies. Plesiomorphic features are also the absences of nuclear envelope, centrioles and microtubules.

FIG. 1. — Transmission electron micrographs of spermatozoa. **a:** *Penaeus japonicus*; **b:** *Parapeneus longirostris*; **c:** *Sicyonia carinata*; **d:** *Aristeus antennatus*; **e:** *Aristaeomorpha foliacea*; **f:** *Solenocera membranacea*; **g:** *Sergestes arcticus*. Scale bars: 1 µm. a: acrosome, ac: acrosomal cap, c: cytoplasm, m: mitochondria-like bodies, n: nuclear region, p: protuberance of the acrosomal cap, s: spike, *: subacrosomal region.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Family Solenoceridae. The spermatozoon of *Solenocera membranacea* is similar to that of Penaeidae in general morphology (Figs 1f, 2d), though it shows conspicuous differences with regard to the other spiked dendrobranchiate sperm. The contents of the acrosomal vesicle are homogeneously electron-dense and the cap appears asymmetrical in sagittal sections, one of its lateral expansions projecting further than the other. Another distinctive feature of the *S. membranacea* spermatozoon is that the plasma membrane becomes separated from the anterior acrosome membrane, the intervening space being occupied by part of the cytoplasmic mass. The perinuclear cytoplasm is rather amorphous, though parallel lamellae and mitochondria-like bodies may be recognized. It is thick under the lateral edges of the acrosomal cap and grows thinner at the posterior part of the sperm. Anteriorly, it forms a thin band separating the scarce subacrosomal substance from the finely filamentous chromatin, a feature that recalls that observed in the penaeid *Parapeneus longirostris* [44].

Family Sergestidae. The sperm of *Sergestes arcticus* are simple, spheroidal or slightly ellipsoidal cells which much resemble those found in *Aristaeomorpha foliacea*. They consist of a central, non-membrane bound nuclear region and surrounding cytoplasm (Figs 1g, 2a). The finely filamentous chromatin mass is encircled by a thin cytoplasmic band that mainly contains densely-packed electron-clear vesicles and a few mitochondria-like bodies. Occasionally, the cytoplasm encloses lipid-like, highly osmiophilic inclusions. At some points, the cytoplasmic band may be interrupted, thus allowing a direct contact of the nucleoplasm with the plasma membrane. Acrosome, microtubules and centrioles are absent.

In eucarids absence of the acrosome had been reported only in Euphausiacea [31] and Stenopodidea [19]. Indeed, there appear to be striking resemblances between the spermatozoa of *Sergestes arcticus*, *Aristaeomorpha foliacea* (Fig. 1e, g) and *Euphausia* sp. (see [31]) which very probably are indicative of phylogenetic relationship. These are: (1) central nuclear region consisting of diffuse, finely filamentous chromatin, (2) complete disruption of the nuclear envelope, (3) vesiculate, thin perinuclear cytoplasmic band, (4) absence of centrioles and microtubules, and (5) absence of acrosome. Now the question arises as to the evolutionary meaning of acrosome-less spermatozoa within Dendrobranchiata. Has this condition been acquired secondarily or is it a primitive one? This subject is discussed below.

Sperm phylogenetic relationships within Dendrobranchiata and between Dendrobranchiata and other Eucarida

The present survey suggests as clear dendrobranchiate spermatozoal symplesiomorphies: (1) complete loss of the nuclear envelope, (2) filamentous chromatin, (3) absence of centrioles, (4) absence of radial (stellate) arms. The plesiomorphic perinuclear distribution of the cytoplasm does not occur in *Aristeus antennatus*; in this species, the cytoplasm forms a collar between the acrosome and nuclear region. Whether the acrosome-less condition of *Aristaeomorpha foliacea* is an apomorphic character or, in contrast, a plesiomorphy, is a matter that remains to be established when more data are available. Nonetheless, the finding of similar, acrosome-less sperm patterns in euphausiids (*Euphausia* sp.) [31], stenopids (*Stenopus hispidus*) [19], sergestids (*Sergestes arcticus*) and aristeids (*Aristaeomorpha foliacea*) appears to point to its plesiomorphy. Although the loss of the acrosome is a repeated event throughout evolution of the crustacean sperm [26], the assumption of sperm originally endowed with an acrosome would suppose the highly improbable independent loss of the acrosome in several separate lineages of the eucarid tree (Fig. 2).

According to JAMIESON [31], "the malacostracan acrosome is a new development, in view of evidence that their acrosome originates from the endoplasmic reticulum and not, as is usual, from the Golgi." Certainly, several studies have demonstrated that the acrosomal structures in Decapoda derive from, or in association with, cisternae of the endoplasmic reticulum itself or of its specialized portion constituting the nuclear envelope [1-3, 14, 23, 35, 38, 40, 43, 44, 46, 49, 53, 55, 56, 58, 60]. Consequently, it can be said that the mechanisms involved in differentiation of the acrosomal structures are somehow plesiomorphic. It is not known whether similar mechanisms take place during spermiogenesis in euphausiids, sergestids, stenopids and acrosome-less aristeids (as they do in fact in *Aristeus antennatus*). If so, it is to be assumed that the capacity to build acrosomes with the involvement of endoplasmic reticulum membrane systems (irrespective of the appearance or not of a distinct acrosome in mature sperm) was present in ancestors of eucarids before separation of euphausiids and decapods. Such a widely shared mechanism of acrosome formation is consistent with the occurrence of apparent homologies in the acrosomes of as distant taxa as the Penaeidae and the Brachyura [46]. In contrast, the caridean spike, in spite of resulting in a sperm pattern closely resembling the peneid-sicyoniid-solenocerid one, is not to be phylogenetically related to the dendrobranchiate spike [44]. Definitely, comparative sperm ultrastructural studies argue against a monophyletic "Natantia", as BURKENROAD [8] conjectured more than a decade ago.

If, as appears plausible (see above), the absence of an acrosome is plesiomorphic, then the spiked acrosome of dendrobranchiates would be a synapomorphy of the families Penaeidae, Sicyonidae and Solenoceridae, whereas the sperm of the Sergestidae and Aristeidae should be considered as more primitive, that of *Aristeus antennatus* showing secondary (thus apomorphic) acquisition of the acrosome independent of the evolutionary line leading to the other acrosome-possessing dendrobranchiate spermatozoa. Taking into account the report, albeit requiring confirmation, that euphausiid spermatozoa [31] are similar to those of *Sergestes arcticus* and *Aristaeomorpha foliacea* (Figs 1e, g, 2a, b), occurrence of a plesiomorphic acrosome-less sperm is congruent with the statement of BURKENROAD [8] that the ancestors of the Decapoda were more euphausiid-like than the modern forms. According to this, the primitive eucarids could have euphausiid-like sperm, euphausiids, sergestids, aristeids and stenopids having retained this pattern. Among Aristeidae, some representatives (*A. antennatus*) might well have recreated a spheroidal acrosome with no ultrastructural resemblance to the acrosome of any of the other known dendrobranchiates, the sperm becoming arranged into a reptant-like pattern (although retaining the plesiomorphic absences of arms, microtubules and nuclear envelope, and therefore with no apparent direct phylogenetic relation to reptants) which represents an independent evolutionary line (see Fig. 2).

Spermiocladistic support for the statement of FELGENHAUER & ABELE [17] that the Caridea and Stenopodidea derive from ancestral reptants would necessitate further research. Derivation of carids from primitive thalassinoids is not congruent with most recent observations on spermatozoal ultrastructure by TUDGE [this volume], unless important deviations (= apomorphies) from the reptant ground plan be assumed, namely the loss of the membrane-bound acrosome and of microtubule-containing radial arms, as well as the independent development of a non-membrane bound spike [2, 3, 16, 18, 38, 42, 52, 54, 56, 57] that acts in a distinct and very particular manner during fertilization [4, 41]. These typically caridean characteristics confirm a sperm pattern that represents a fairly distinct, clearly identifiable evolutionary trend within the Decapoda.

The occurrence of either a complete or a discontinuous double-membrane nuclear envelope, partially invested by the plasma membrane, as well as the occasional presence of centrioles at the base of the acrosome, are shared by carids and reptantians, these features supporting a certain unity of both groups. However, the supposed reptantian origins of stenopodideans [17] are disputed by the ellipsoidal, arm-less and acrosome-less form of the spermatozoon of *Stenopus hispidus* [19], which is also characterized by having a lamellar body located against the plasma membrane at one side of the sperm cell (a structure that strongly suggests reminiscence of the well developed membrane system associated with proacrosomal vesicles in decapods). At first glance, this sperm morphology would place the stenopodideans close to the euphausiids, hence suggesting an early separation of Stenopodidea from the reptantian-caridean stem just above the origin of the Dendrobranchiata and before appearance of the acrosome and of appendages in decapod spermatozoa (see Fig. 2).

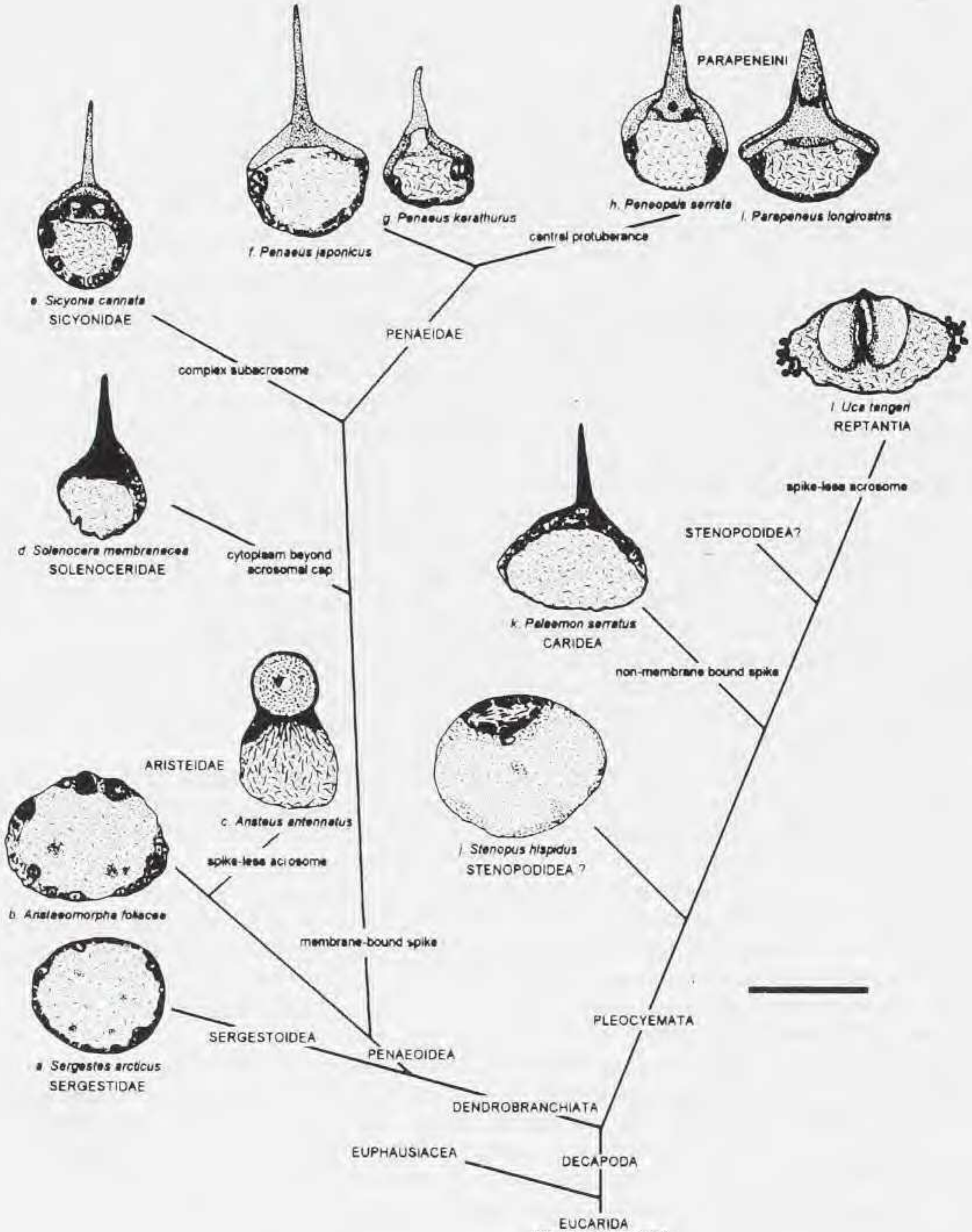

FIG. 2. — Intuitive phylogenetic tree of the sperm of the Eucarida where the different dendrobranchiate spermatozoal patterns are represented. Figures b, c, e-k have been prepared from drawings or micrographs which appeared in [19, 44-48, 52]; a and d are original. Scale bar: 8 µm for k, 4 µm for the others.

Source : MNHN, Paris

Figure 2 represents a tentative phylogenetic tree which attempts to reconcile the current knowledge of spermatozoal ultrastructure in eucarids with phyletic relationships suggested recently by reputable authors [7, 17, 26, 31, 36, 59]. Although the phylogram is necessarily provisional owing to the limited number of studies available, the most important spermatozoal evolutionary trends are represented in it. In the proposed sperm phylogram, separation of the Euphausiacea is followed by a node grouping the Decapoda, with two distinct evolutionary lines, one of which leads to Dendrobranchiata and the other to Pleocyemata. It is believed that the decapod sperm were originally devoid of an acrosome, a condition that was retained in Sergestidae as well as representatives of the family Aristeidae (*Aristaeomorpha foliacea*). However, another aristeid (*Aristeus antennatus*) has a spermatozoon supplied with an apomorphic membrane-bound acrosome that resembles the sperm of *Panulirus* spp. owing to concurrence of several parallelisms rather than to phylogenetically-based shared features. The three other families of the Dendrobranchiata have in common spermatozoa which share a synapomorphic membrane-bound acrosomal spike. From the node uniting these non-aristeid sperm, the first branch to emerge is represented by the spermatozoon of *Solenocera membranacea*, which shows an asymmetrical acrosomal cap and separation of the plasma and anterior acrosome membranes, allowing part of the cytoplasm to "leak" beyond the acrosomal cap. Finally, Sicyonidae and Penaeidae appear as two aligned groups, the sperm of which are easily distinguishable by the highly complicated, apomorphic subacrosomal region present in sicyoniids, in contrast to the simple one of penaeids. In the Penaeidae, two distinct sperm types have been recognized on the basis of the presence (*Parapeneus longirostris* and *Peneopsis serrata*) or absence (*Penaeus* spp.) of a central protuberance at the concave side of the acrosomal cap. This dendrobranchiate sperm phylogenetic arrangement is in agreement with the close interrelation that Burkenroad [8] suggests between penaeids and sicyoniids. However, with our limited information, no spermatozoal evidence has been found to ally, as he claims, aristeids and solenocerids. On the contrary, the spermatozoon of *S. membranacea* resembles the Penaeidae-Sicyonidae sperm rather than any of the known Aristeidae sperm types.

The Pleocyemata lineage (Fig. 2) would first include acrosome-less sperm forms, such as those present in *Stenopus hispidus*. Therefore, a logical phylogenetic sequence would suggest a first offshoot leading to Stenopodidea in a scheme that is congruent with the phylogram of FELGENHAUER & ABELE [17]. However, another spermatologically plausible, albeit less probable, arrangement following the more recent cladograms of SCHRAM [59] and KIM & ABELE [36], would place the offshoot of Stenopodidea between the branches leading to Caridea and Reptantia.

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